Acta Odontologica Scandinavica, 2009; 67: 321–332

informa
healthcare

REVIEW ARTICLE

Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis

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Abstract

Objective. This systematic review with meta-analyses sought to answer the following question: "Does CPP-ACP [casein phosphopeptide-amorphous calcium phosphate], when introduced into the oral environment, provide any caries-preventive benefit superior to that of any other intervention or placebo?" ***Material and methods.*** Seven electronic databases were searched for trials relevant to the review question. Twelve articles were accepted after application of inclusion and exclusion criteria. ***Results.*** Of the accepted articles, five *in situ* randomized control trials (RCT) could be pooled for meta-analyses. During the short-term (7–21 days) *in situ* trials, participants wore appliances containing enamel slabs that were analyzed in the laboratory after exposure to CPP-ACP. The pooled *in situ* results showed a weighted mean difference (WMD) of the percentage remineralization scores in favor of chewing gum with 18.8 mg CPP-ACP as compared to chewing gum without CPP-ACP (WMD −8.01; 95% CI: −10.54 to −5.48; $p=0.00001$), as well as compared to no intervention (WMD −13.56; 95% CI: −16.49 to −10.62; $p=0.00001$). A significant higher remineralization effect was also observed after exposure to 10.0 mg CPP-ACP (−7.75; 95% CI: −9.84 to −5.66; $p=0.00001$). One long-term *in vivo* RCT (24 months) with a large sample size ($n=2720$) found that the odds of a tooth surface's progressing to caries was 18% less in subjects who chewed sugar-free gum containing 54 mg CPP-ACP than in control subjects who chewed gum without CPP-ACP ($p=0.03$). ***Conclusion.*** Within the limitations of this systematic review with meta-analysis, the results of the clinical *in situ* trials indicate a short-term remineralization effect of CPP-ACP. Additionally, the promising *in vivo* RCT results suggest a caries-preventing effect for long-term clinical CPP-ACP use. Further randomized control trials are needed in order to confirm these initial results *in vivo.*

Key Words: *Caries, CPP-ACP, meta-analysis*

Introduction

The potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to promote remineralization and inhibit demineralization of hard tooth tissue has been observed in laboratory and animal studies [1,2] and *in situ* studies covering human subjects [3,4]. Explanation of this potential has been based on the ability of casein phosphopeptide (CPP) to stabilize calcium phosphate by binding amorphous calcium phosphate (ACP) and thus forming CPP-ACP clusters [5]. These CPP-ACP clusters act as a calcium and phosphate reservoir that attaches itself to dental plaque and tooth surfaces. On acid challenge, the attached CPP-ACP releases calcium and phosphate ions, thus maintaining a supersaturated mineral environment, thereby reducing demineralization and enhancing remineralization [6–8]. It has been shown that enamel remineralized by CPP-ACP is relatively more acid-resistant than normal tooth enamel [3,7].

The most commonly tested (and used) mode of CPP-ACP application in the human oral environment is via sugar-free sorbitol or xylitol-based chewing gum [3,4,7]. Other vehicles include milk [9], mouth-rinses [10], lozenges [11,12], and dental cream [13]. A recent systematic review, which covered a number of published trials on this topic, reported on the clinical efficacy of casein derivatives, including CPP-ACP [14]. The investigated outcomes included the efficacy of CPP-ACP for caries prevention (10 studies), treating dry mouth (1 study), and treating dentin hypersensitivity (1 study). The authors found "insufficient clinical trial evidence (in quantity, quality or both) to make a recommendation

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(Received 7 March 2009; accepted 3 July 2009)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350903160563

regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP, in preventing caries *in vivo* and in treating dentin hypersensitivity or dry mouth". This conclusion was based on the authors' assessment of each included trial, using a PICOS (patient; intervention; controls; outcome; study authors' conclusions) format and a qualitative synthesis of the included articles. However, the disadvantage of qualitative synthesis in systematic reviews is that bias may be introduced if the outcomes of some studies are inappropriately stressed over others [15]. The advantages of meta-analysis over qualitative synthesis is that it provides the opportunity to identify a treatment effect as statistically significant ($p < 0.05$) and to improve estimation of the effect by quantifying its outcome, thus making its estimation more precise [15]. Therefore, while methodological weaknesses limit what can be inferred in terms of efficacy, the cumulative weight of evidence (as highlighted, where possible, in a meta-analysis) provides a more objective assessment of a systematic analysis of the literature.

The inconclusive findings of the Azarpazhooh & Limeback systematic review [14] regarding the outcome "caries prevention" (7 trials favored CPP-ACP in comparison to control, 2 studies found no additional benefit and 1 study had contradictory findings) might have been very different if a meta-analysis of trials reporting on the same outcome had been attempted. This has been the case in a number of systematic reviews where individual studies have had varied outcomes, but the cumulative weight of the evidence (elicited through pooling together trials with similar outcomes) has been found to be conclusive for that particular outcome [16–18]. Thus, this systematic review with meta-analysis sought to answer the following question: "Does CPP-ACP, when introduced into the oral environment, provide any caries-preventive benefit superior to that of any other intervention or placebo?"

Material and methods

Search strategy

The literature search covered the electronic databases: Biomed Central; Cochrane oral health reviews; Cochrane library; Directory of open access journals (DOAJ); PubMed; Science Direct; Research findings electronic register – ReFeR. In order to search databases, strings of search terms, consisting of relevant text words and boolean links, were constructed. The string of English search terms: "MI Paste OR Recaldent OR casein phosphopeptide-amorphous calcium phosphate OR CPP-ACP OR tooth mousse" was used. All publications listed between the earliest publication year of each particular database and 31 August 2008 were included in the search.

Inclusion and exclusion criteria

Publications were selected from the search results if their titles/abstracts were relevant to the review objective and the articles were published in English. Additionally, since the review question dealt with a therapeutic intervention, each included study had to be either a clinical trial (randomized or quasi-randomized; *in situ* or *in vivo*) or a systematic review (with or without meta-analysis) of published trials that reported on the efficacy of CPP-ACP in any mode of delivery. The rationale behind using broad-based inclusion criteria was that the reviewers could scan the reference sections of all studies on casein derivatives to try to identify additional trials that could be considered for possible inclusion in this review. Case reports, editorials, case series, *in vitro* studies, studies that included animal (bovine) tissue, and review papers that were not considered systematic reviews, were excluded. Where only a relevant title without a listed abstract was available, a full copy of the publication was assessed for inclusion. In accordance with published recommendations [19], included articles were reviewed independently by two reviewers (V.Y. and S.M.). Disagreements were resolved through discussion and consensus. Where multiple reports covered the same trial, the one covering the longest period and lacking the exclusion criteria was accepted.

Quality of studies

The quality assessment of the included trials was undertaken independently by two reviewers (V.Y. and S.M.) and piloted using trials not included in this review. Quality assessment rating, scored by both reviewers, was derived by consensus. Four commonly accepted quality criteria [20–22] relating to the internal validity of the trials were examined:

1. Generation of randomization sequence, recorded as: (A) adequate (e.g. computer-generated random numbers, table of random numbers), (B) unclear, (C) inadequate (e.g. case record number, date of birth, date of administration, alternation).
2. Allocation concealment, recorded as: (A) adequate (e.g. central randomization, sequentially numbered sealed opaque envelopes), (B) unclear, (C) inadequate (e.g. open allocation schedule, unsealed or non-opaque envelopes).
3. Blind outcome assessment, recorded as: (A) yes, (B) unclear, (C) no, (D) not used/possible.
4. Completeness of follow-up (whether a clear explanation existed for withdrawals and drop-outs in each treatment group), assessed as: (A)

yes (drop-outs less than 30%), (B) yes (drop-outs more than 30%), (C) no explanation.

Data extraction and meta-analysis

The primary outcome measure was caries prevention reported in accordance with the requirements listed below.

a. An improvement in DMFT/DMFS/DFS scores with standard deviations (SDs) or 95% confidence intervals (CI) or standard errors of the mean (SEM)

The measures sought for pooling of data for meta-analyses were the mean DMFT/DMFS/DFS scores with SDs. If the SD was not reported, this was calculated from the 95% CIs or the SEM scores. Where no SD score was included or could be calculated, the paper was excluded.

b. A percentage remineralization (%R) with SDs (increase or decrease)

Since this is a continuous variable, pooling of data (for meta-analysis) from included trials was undertaken using the Cochrane RevMan, v. 4.2, software package. The differences in the %R scores were calculated as follows: %R control group – %R treatment group. A negative score would imply benefit (more remineralization would have occurred after exposure to CPP-ACP in the treatment group).

c. A change in lesion depth (either increase or decrease)

Two reviewers (V.Y. and S.M.) independently extracted data from the accepted articles using a pilot-tested data extraction form. Disagreements between reviewers during data extraction were resolved through discussion and consensus. The results of the included studies were treated as continuous data. Trials were assessed for their clinical and methodological heterogeneity, following Cochrane guidelines [23], and were considered homogenous if they had not differed substantially in the following clinical and methodological aspects: type of delivery agent used (e.g. chewing gum), type of control material (e.g. chewing-gum without CPP-ACP; no intervention), frequency of application/use, CPP-ACP concentration (e.g. 18.8 mg; 10.0 mg) and outcome measure (e.g. %R). Clinically and methodologically homogenous trials were combined and analyzed separately in subgroups, for which the random effects model of the meta-analysis software, RevMan v. 4.2, was used. Studies were assigned a Mantel-Haenszel weight in direct proportion to their sample size. Differences between groups for each of the assessed pooled outcomes were reported in the form of weighted mean differences (WMDs) and their respective 95% confidence intervals (CIs). Forest plots were used to graphically illustrate results of subgroup meta-analyses undertaken. For trials where pooling of data was not possible, mean differences (MDs) were calculated to reflect differences in the treatment and control groups.

Results

The initial search in the various electronic databases, using the key words listed in the search strategy, yielded 3459 articles. Application of the broad-based inclusion criteria significantly reduced these to 5 reviews and 30 clinical studies. Of the 35 articles, 23 were not considered after application of the exclusion criteria (Figure 1). Table I provides a summary of reasons for their exclusion. Eleven trials [3,4,6–10,12,13,24,25] and one systematic review [14] were finally accepted for this review (see Table II).

Appraisal and quality assessment of included studies

Table II provides a summary of included trials in a PICOS (Population, Intervention, Comparative intervention or control, Outcomes, Study design) format and Table III reports on a quality assessment of included trials. Of the 11 trials, 9 [3,6–10,12,24,25] were double-blinded, *in situ*, randomized controlled trials (RCT) with a crossover component. Most had small sample sizes ($n < 15$). However, two [6,24] had sample sizes of 30 and short follow-up periods (on average 14 days, with the exception of one trial [25], which had a follow-up of 21 days). Two trials [4,13] were RCTs with longer follow-up periods of 12, and 24 months respectively. In terms of quality assessment, all included trials, except two [9,13] (Allocation concealment was unclear – "B"), scored "A" (adequate) for Randomization, Allocation concealment and Blinding. All of those included provided information on sample sizes, loss-to-follow-up rate and follow-up periods. For the pooled meta-analysis, all of the included papers were rated "A" for Randomization, Allocation Concealment, Blinding, and Drop-outs. However, all of the studies included for the meta-analysis were *in situ* in study design and were of short-term (7–21 days) duration. During these *in situ* trials participants wore appliances containing enamel slabs that were analyzed in the laboratory after exposure to CPP-ACP.

Pooling of data for meta-analyses

Only trials that were considered clinically and methodologically homogenous and reported on similar outcomes were pooled for meta-analyses. For this review, three subgroups were analyzed (Figures 2–4).

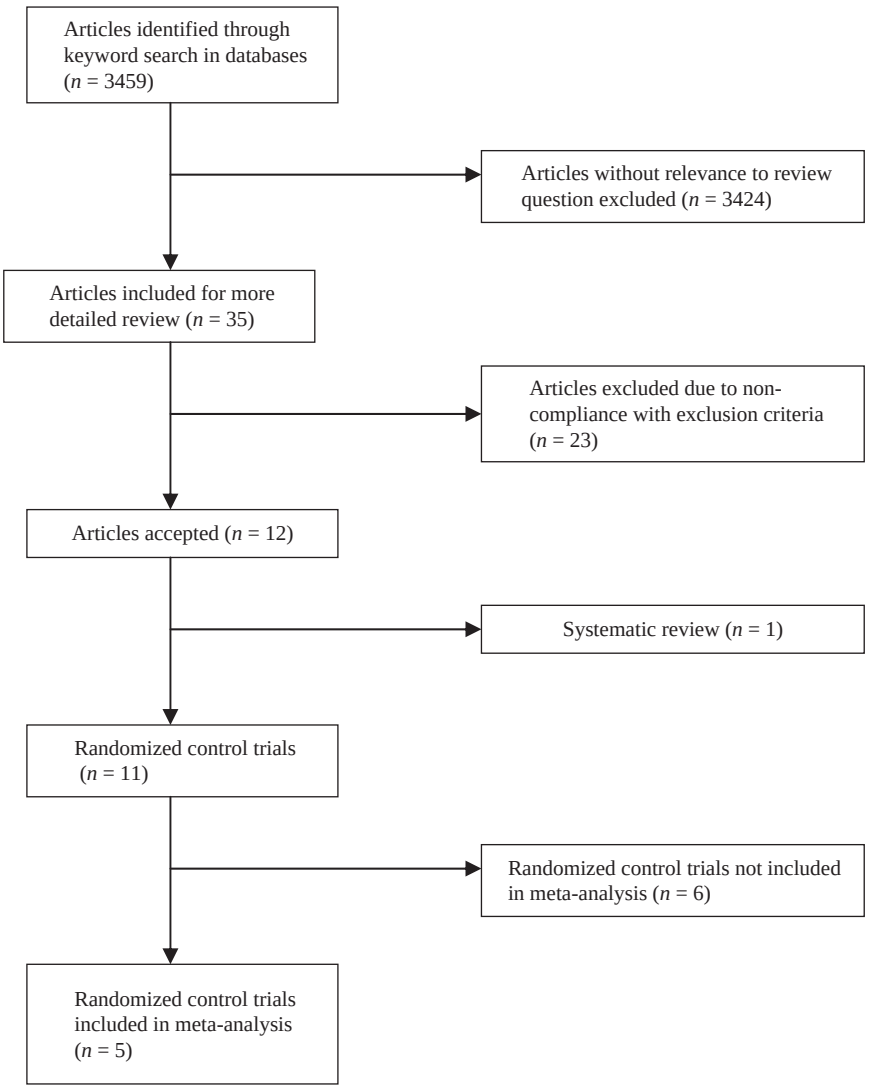

Figure 1. Flow chart of article review and meta-analysis.

Figure 2 provides information on the cumulative weight of evidence for the caries-preventive effect of 18.8 mg CPP-ACP (delivered via sugar-free gum) when compared to that of sugar-free gum without CPP-ACP. Four data sets, from 3 trials (Figure 2) with individual weighted mean differences (WMDs) for control and intervention groups, contributed to the overall effect. This found in favor of groups that chewed gum containing 18.8 mg CPP-ACP (WMD −8.01; 95% CI: −10.54 to −5.48; $p < 0.00001$). All the trials had a crossover *in situ* design with a 14-day test period followed by 7-day washout periods between interventions. The outcome of interest (caries prevention) was reflected as the percentage remineralization (%R). Similarly, when results for those receiving a lowered dosage of 10.0 mg CPP-ACP were compared with control groups (Figure 3), the cumulative WMD (WMD −7.75; 95% CI: −9.84 to −5.66; $p < 0.00001$) favored the group exposed to 10.0 mg CPP-ACP. In the 3rd subgroup analysis (Figure 4), groups whose interventions contained 18.8 mg CPP-ACP, delivered via sugar-free gum (slab or pellet) or lozenges, were compared with those receiving no intervention. Data for this meta-analysis were obtained from two trials [3,24]. There was a significant improvement in the percentage remineralization (%R) in groups exposed to 18.8 mg CPP-ACP over the study period, when compared to the no-treatment groups (WMD −13.56; 95% CI: −16.49 to −10.62; $p < 0.00001$).

The mean differences (MDs) for studies where the data could not be pooled for meta-analysis were also calculated (where possible) to reflect the size of the treatment effect disparity between the intervention (CPP-ACP) and control groups. In the Itthagarun et al. trial [25], three types of chewing gum containing 30 mg urea, 30 mg urea + 25 mg dicalcium phosphate dehydrate or 30 mg urea + 47 mg CPP-ACP gum were tested in an *in situ* crossover trial consisting of 12 subjects. Only 9 subjects completed the trial and the caries remineralizing effect of CPP-ACP versus 30 mg urea (reported as change in lesion

Table I. Excluded articles and main reasons for exclusion.

Authors	Reason for exclusion
Aimutis WR 2004 [42]	Narrative review
Ardu S et al. 2007 [34]	Case report
Cochrane NJ et al. 2008 [31]	*In vitro* study
Hay et al. 2005 [11]	Investigated casein derivatives such as calcium phosphate but not CPP-ACP
Hicks J et al. 2004 [41]	Narrative review
Mazzaoui SA et al. 2003 [43]	*In vitro* study
Milnar FJ et al. 2007 [36]	Case report
Oshiro M et al. 2007 [2]	Study on animal tissues
Pai D et al. 2008 [29]	*In vitro* study
Piekarz C et al. 2008 [30]	*In vitro* study
Rahiotis C et al. 2007 [35]	*In vitro* study
Ramalingam L et al. 2005 [40]	*In vitro* study
Reynolds EC 1997 [5]	*In vitro* study
Reynolds EC 1998 [44]	Narrative review
Schirrmeister JF et al. 2007 [45]	Study on animal tissues
Slayton RL 2006 [47]	Narrative review
Sudjalim TR et al. 2006 [38]	Narrative review
Sudjalim TR et al. 2007 [37]	*In vitro* study
Tantbirojn D et al. 2008 [32]	*In vitro* study
Vlacic J et al. 2007 [33]	Case report
Yamaguchi K et al. 2006 [39]	Study on animal tissues
Yamaguchi K et al. 2007 [1]	Study on animal tissues
Zero DT 2006 [46]	Narrative review

depth) favored CPP-ACP (MD −16.6; 95% CI −30.37 to −1.95; $p=0.03$). However, when CPP-ACP was compared with another casein derivative, 25 mg dicalcium phosphate dehydrate, no significant differences were noted for the MDs; implying an equivalent treatment effect (MD −1.0; 95% CI%: −14.58 to 12.58; $p=0.89$). The Reynolds et al. [6] trial compared the remineralizing effect of CPP-ACP in sugar-free gum against other forms of calcium in gum in 30 adults in a crossover *in situ* study. The MD for 9.5 mg CPP-ACP gum versus gum containing $CaHPO_4/CaCO_3$ favored CPP-ACP (MD −7.00; 95% CI: −5.94 to −8.06; $p<0.00001$). Similar results were obtained when CPP-ACP gum (either in pellet or slab form) was compared to gum with $CaCO_3$ only.

In another trial, also by Reynolds et al. [10], 14 subjects were given a toothpaste slurry containing (1) placebo, (2) 1100 ppm fluoride, (3) 2800 ppm fluoride, (4) 2% CPP-ACP, or (5) 2% CPP-ACP+1100 ppm fluoride, in a 14-day crossover trial, with 7-day washouts between treatments. The MDs of the percent remineralization, reported as an outcome between 2% CPP-ACP+1100 ppm fluoride and 1100 ppm fluoride toothpaste, favored the CPP-ACP group (MD −12.80; 95% CI −9.54 to −16.06; $p<0.00001$). Similar significant MDs were obtained when 2% CPP-ACP+1100 ppm fluoride was compared against all the other products used in this study. In one trial, CPP-ACP was added to bovine milk and its remineralizing effect was investigated by testing 2.0 and 5.0 g/l CPP-ACP in milk against the placebo (milk with no added CPP-ACP) [9]. The milk with 5.0 g/l CPP-ACP had significantly higher %R mean scores than 2.0 g/l CPP-ACP and no CPP-ACP-containing milk (11.4 versus 7.8 versus 4.6, respectively).

One trial reported that the odds of a tooth surface's progressing to caries in subjects who chewed sugar-free gum containing 54 mg CPP-ACP was 18% less than in controls who chewed gum lacking CPP-ACP ($p=0.03$) [4]. The large sample size ($n=2720$ children) and long follow-up (24 months) used in this RCT were unique in terms of CPP-ACP efficacy trials.

Andersson and colleagues [25] compared the remineralizing effect of dental cream containing CPP-ACP in 13 subjects using cream for 3 months, followed by 3 months' use of 1100 ppm fluoride toothpaste. These completed orthodontic treatment and were debonded with a control group ($n=13$) that used only 0.05% NaF mouthwash+1100 ppm fluoride toothpaste over a 6-month period [13]. The outcome of interest was the regression of white spot lesions. Although both groups showed significant improvement at 12-month observation, the number of white spot lesions that had completely disappeared at 12 months was significantly greater in the CPP-ACP group (63% versus 25%, respectively; $p<0.05$).

Discussion

The primary objective of this systematic review with meta-analysis was to determine, through studying published clinical trials, the caries-preventive effect of CPP-ACP. No attempt was made to search for trials in the grey literature or non-English databases and papers published in a language other than English were excluded. Although this introduced an element of bias, the searched databases covered the majority of the biomedical published literature and also included non-English papers. However, no non-English papers or abstracts were identified in the search strategy used for this review.

For all of the pooled meta-analyses reported (Figures 2–4), lesions exposed to CPP-ACP (18.8 mg or 10.0 mg) were found to have a more significant improvement in remineralization than control lesions that were not exposed to CPP-ACP. All the studies used in the meta-analyses were *in situ* RCTs with a crossover component. The obvious limitation of requiring participants to wear appliances containing enamel slabs that were analyzed in a laboratory after exposure was that the length of exposure was relatively short (less than 15 days for most trials). (Slabs were sectioned and the percent mineral profile of each enamel block's demineralization and remineralization lesion was compared with that of the median sound enamel between the lesions of the same section via

Table II. Details of included studies.

Author/year	Population	Intervention	Comparative intervention/controls	Outcome/s	Study design
Iijima et al. 2004 [7]	10 healthy subjects (mean age 32.3; SD $\pm$7.9 years).	Two gum treatments: 1. Dental chewing gum in slabs containing CPP-ACP (18.8 mg). 2. Sugar-free gum in slabs without CPP-ACP.	Crossover design with 14-day test period followed by 7-day washouts between interventions. *In vitro* acid challenge of enamel slabs done for 8 and 16 h.	% subsurface remineralization [%R] (CPP-ACP vs Control). Three measures reported: 1.%R with no acid challenge (17.88 $\pm$0.97 vs 9.02 $\pm$0.74). 2.%R after 8 h acid challenge (12.43 $\pm$0.90 vs 3.12 $\pm$0.88). 3.%R after 16 h acid challenge (10.40 $\pm$1.19 vs 1.08 $\pm$1.02.	Double-blinded *in situ* and *in vitro* RCT with crossover.
Itthagarun et al. 2005 [25]	12 healthy subjects (5 males, 7 females; age range 20–47 years).	Three types of sugar-free gum containing: 1. 30 mg urea. 2. 30 mg urea + 25 mg dicalcium phosphate dehydrate. 3. 30 mg urea + 47 mg CPP-ACP.	Crossover design with 21-day test period for each type of gum followed by 5-day washouts after each test period.	Two outcomes reported: 1. Mean % change in lesion depth of the samples. 2. Mean % change in mineral content of the samples.	Double-blinded *in situ* RCT with crossover.
Shen et al. 2001 [24]	30 healthy subjects (30 $\pm$7; 33 $\pm$7 and 34 $\pm$6 years).	Three types of gum: 1. Sorbitol-based pellet gum containing four different doses of CPP-APP. 2. Sorbitol-based slab gum containing four different doses of CPP-APP. 3. Xylitol-based pellet gum containing four different doses of CPP-APP. Doses in mg of CPP-ACP were 0, 0.19, 18.8 and 56.4 mg.	Crossover design with 14-day test period for each type of gum followed by at least 1 week washout period between interventions.	% subsurface remineralization (%R).	Double-blinded *in situ* RCT with crossover.
Reynolds et al. 2003 [6]	30 healthy adults (age range 22–44 years).	Consisting of two parts: A. Mouth-rinse trial with four interventions tested: 1. 2% CPP-ACP. 2. 6% CPP-ACP. 3. Calcium + phosphate slurry mixed as mouth rinse. 4. De-ionized water. B. Chewing gum trial in two parts: 1. Gum either in pellet or slab form contained a calcium additive $CaCO_3$ or $CaHPO_4/CaCO_3$ or CPP-ACP (two types of gum with three different additives). 2. Subjects chewed gum pellets containing 9.5 mg CPP-ACP for 4 days without using any other oral hygiene methods.	Mouth-rinse trial: crossover in design; washout period 4 weeks between treatments. Chewing gum trial: crossover in design; no washout period stated; *in situ* study.	For mouthrinse trial: calcium and phosphate levels in supragingival plaque. For chewing gum trial: % subsurface remineralization (%R) and level of CPP in plaque.	Double-blinded RCT; crossover in design; chewing gum has *in situ* component.

Table II (*Continued*)

Author/year	Population	Intervention	Comparative intervention/controls	Outcome/s	Study design
Cai et al. 2007 [3]	10 healthy subjects (7 males, 3 females; age range 23–46 years).	Three treatments: 1. Sugar-free pellet gum containing 20 mg citric acid + 18.8 mg CPP-ACP. 2. Gum with 20 mg citric acid. 3. Gum with neither citric acid nor CPP-ACP.	Crossover trial with 2-week treatment periods followed by 7-day washout.	1.% Subsurface remineralization. 2.% Remineralization after 16-h acid challenge.	Double-blinded *in situ* RCT with crossover.
Walker et al. 2006 [9]	10 healthy adults.	Three treatments: 1. 200 ml milk containing 2.0 g CPP-ACP/l. 2. 200 ml milk containing 5.0 g CPP-ACP/l. 3. 200 ml milk containing no CPP-ACP.	Crossover trial with 15-day treatment periods (200 ml milk consumed over 60 s) followed by 7-day washout.	% subsurface remineralization (%R).	Double-blinded *in situ* RCT with crossover.
Cai et al. 2003 [12]	10 healthy subjects (6 males, 4 females; mean age 34 ± 6.6 years).	Four treatments consisting of 1.75 g lozenge with: 1. 18.8 mg CPP-ACP. 2. 56.4 mg CPP-ACP. 3. No CPP-ACP No lozenge; no treatment; control.	Crossover design with 14-day test period for each type of lozenge ($4\times$ daily use) followed by at least 1 week washout period between interventions.	% subsurface remineralization (%R).	Double-blinded *in situ* RCT with crossover.
Manton et al. 2008 [8]	10 healthy subjects (6 males, 4 females).	Three types of gum: 1. Sorbitol/xylitol-based 2.0 g slab gum containing no CPP-APP. 2. Sorbitol/xylitol-based 1.5 g pellet ($\times 2$) gum containing no CPP-APP. 3. Two gum pellets containing 10 mg CPP-ACP.	Crossover design with 14-day test period for each type of gum (4 times daily use) followed by 7-day washout period between interventions.	% subsurface remineralization (%R).	Double-blinded *in situ* RCT with crossover.
Morgan et al. 2008 [4]	2720 healthy children randomized into test ($n = 1369$) and control ($n = 1351$).	Gum with 54 mg CPP-ACP chewed 3 times daily for 10 min per session. 926 children completed trial. 439 dropped out.	Sorbitol-based gum- chewed 3 times daily for 10 min per session. 894 children completed trial. 452 dropped out.	Caries progression or regression at 24 months. Approximal caries diagnosed via digital bitewing X-rays.	Double-blind RCT.
Reynolds et al. 2008 [10]	14 healthy subjects (7 males, 7 females; age range 21 to 45 years).	Two RCTs: A. Three mouthrinses containing either: 1. 2% w/v CPP-ACP + 450 ppm F as NaF in de-ionized water. 2. 450 ppm F as NaF in de-ionized water. 3. Placebo control rinse as de-ionized water. B. Toothpaste trial, each toothpaste slurry containing either: 1. Placebo. 2. 1100 ppm F as NaF. 3. 2800 ppm F as NaF. 4. 2% CPP-ACP. 5. 2% CPP-ACP + 1100 ppm F as NaF.	A. Crossover trial with 15 ml rinses 3 times daily for 4 days and once on the fifth day. No other oral hygiene method used in test period. Washout period was 4 weeks between interventions. B. Crossover trial with 4 rinses per day for 14 days followed by 7-day washouts between interventions. In-vitro acid challenge of enamel slabs done after *in situ* study.	1. Plaque fluoride levels. 2.% subsurface remineralization (%R). 3.% remineralization after acid challenge.	Double-blinded *in situ* and *in vitro* RCT with crossover.

Table II (*Continued*)

Author/year	Population	Intervention	Comparative intervention/controls	Outcome/s	Study design
Andersson et al. 2007 [13]	26 healthy subjects (13 boys, 13 girls; mean age 14.6 years; age range 12–16 years; 60 teeth; 152 white spot lesions on canines and incisors) who were debonded following fixed orthodontic treatment.	Test group consisted of 13 subjects; 70 sites. Treatment: Brushing twice daily with dental cream containing CPP-ACP for 3 months followed by use of 1100 ppm F toothpaste for 3 months.	Control group comprised 13 subjects; 62 sites. Treatment: 0.05% NaF mouthwash +1100 ppm F toothpaste for 6 months.	Regression of white spot lesions diagnosed via visual inspection and laser fluorescence over 1, 3, 6 and 12 months.	RCT.

microradiography.) The *in situ* study design used to determine percentage remineralization is not ideal but can be justified, as the method used to measure the amount of remineralization required sectioning of the enamel. Orthodontic patients with teeth due for extraction would be ideal subjects for trials of this nature. However, the evidence from well-conducted randomized controlled trials [4,13] has added to the weight of evidence showing the effectiveness of CPP-ACP.

The significant results obtained for the meta-analyses, shown in Figures 2–4, suggest that a longer-term exposure to CPP-ACP offers hope of an even greater treatment effect in terms of its caries-preventive efficacy. Indeed, the results of one RCT provide *in vivo* evidence (Table III) that long-term use of CPP-ACP also provides a significant caries-preventive effect in groups who receive this intervention [4]. It must be noted, though, that the children in the test group in this trial were exposed to 54 mg CPP-ACP added to sugar-free chewing gum, which is significantly greater than the 10.0 and 18.8 mg concentrations used in the short-term *in situ* trials (Figures 2–4). One further trial also adds to the weight of evidence supporting the longer-term use of CPP-ACP in patients [13]. In this trial, conducted by authors independently of Reynolds et al. who patented the CPP-ACP technology, significant improvements were noted in both groups, but the number of white spot lesions that had completely disappeared after 12 months was significantly greater in the CPP-ACP group (63% versus 25%, respectively; $p < 0.05$). This randomized control trial provided independent *in vivo* confirmation of the mainly *in situ* findings of Reynolds et al. While the size of the treatment effect was significant, it should be noted that the small sample size ($n = 13$) in the test and control groups could have led to an overestimation of the treatment effect.

The Azarpazhooh & Limeback systematic review [14] reporting on the clinical efficacy of casein derivatives, including CPP-ACP, for the caries prevention, dry mouth and dentin hypersensitivity outcomes, found "insufficient clinical trial evidence" (in quantity, quality or both) on which to base a recommendation regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP, in preventing caries *in vivo* and in treating dentin hypersensitivity or dry mouth" [14]. In the context of the included trials and their search strategy limit (up to October 2007), their conclusions were valid. However, one RCT (published in 2008) significantly contributes to the evidence that shows a longer-term caries-preventive effect of CPP-ACP when delivered in sugar-free chewing gum [4]. The large sample size ($n = 2720$), the long-term follow-up (24 months) and the excellent rating achieved in the quality assessment (Table III) provide good evidence of

Table III. Quality assessment of included studies.

Author/year	Randomization	Allocation concealment	Blinding	Sample size (n)	Drop-outs	Follow-up period
Iijima et al. 2004 [7]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	10	A – None	Crossover design 14 days $\times 2$ with 7×2 day washouts
Itthagarun et al. 2005 [25]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	12	A – 3	Crossover design 21 days $\times 3$ with 5×3 day washouts
Shen et al. 2001 [24]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	10	A – None	Crossover design 14 days $\times 3$ with 7×3 day washouts
Reynolds et al. 2003 [6]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	30	A – None	Crossover design 14 days $\times 3$ with unknown washout period
Cai et al. 2007 [3]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	10	A – None	Crossover design 14 days $\times 3$ with 7×3 day washout period
Walker et al. 2006 [9]	A–Adequate Coded randomization	B–Unclear	A–Yes Double-blind	10	A – None	Crossover design 15 days $\times 3$ with 7×3 day washout period
Cai et al. 2003 [12]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	10	A – None	Crossover design 14 days $\times 4$ with 7×4 day washout period
Manton et al. 2008 [8]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	10	A – None	Crossover design 14 days $\times 3$ with 7×3 day washout period
Morgan et al. 2008 [4]	A–Block Randomization	A–Sealed coded envelopes	A–Yes Double-blind	2720 Test Group ($n=1369$) Control group ($n=1351$)	Test Group 439 Control group 452 B – dropouts >30% (33%)	24 months
Reynolds et al. 2008 [10]	A–Adequate Central randomization	A–Adequate Central randomization	A–Yes Double-blind	14	A – None	Crossover design 14 days $\times 5$ with 7×5 day washout period
Andersson et al. 2007 [13]	A–Adequate Assignment made by use of dice	B–Unclear	A–Blinded examiner	26	A – None	12 months

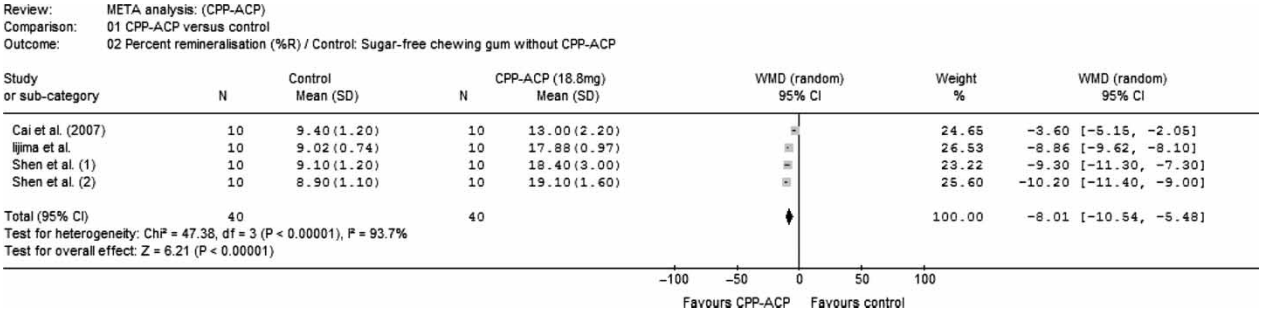

Figure 2. Percentage remineralization (%R) – Subgroup 1: Sugar-free gum with CPP-ACP versus sugar-free gum without CPP-ACP. CI = confidence interval; WMD = Weighted mean difference; N = Sample size.

long-term caries-preventive efficacy. Although the drop-out rate was 33% in this trial (rated "B" in the quality assessment for "Drop-out"), the authors provided detailed reasons for the drop-out rate and this was mainly due to children in the trial moving schools.

The authors of an observational study where the methodological quality of 250 trials from 33 meta-analyses were analyzed to determine the association between methodological quality and estimated treatment effects commented that variables such as random allocation, allocation concealment and blinding were key measures in determining the quality of results reflected in a trial [20]. Random allocation remains the only way to eliminate selection bias [20] and one report [26] warned of potential biases of up to 30% if this is ignored. For allocation concealment and blinding, unclearly concealed trials or trials that were not double-blinded were found to exaggerate the estimates of the treatment effects by up to 30% [20]. Thus, it is clear that systematic reviews which do not include a comprehensive quality assessment of included trials actually create bias in terms of answering their review question, as the weight of the evidence for or against an intervention is intricately linked to the quality of the included studies. In the case of one trial [4], its high quality rating scores, together with the results obtained, provides strong evidence of a long-term caries-preventive effect for CPP-ACP. Moreover, the assertion [14], that the majority of included *in situ* trials were conducted by the group of investigators who patented the CPP-ACP complex (all these trials found in favor of CPP-ACP), creates an impression that the authors of these trials were biased in terms of how they presented their findings [3,6,7,9,12,14,24]. This is misleading, as a quality assessment of these (see Table III) is similar to that of another *in situ* trial [25] by authors who were not part of the group that patented the CPP-ACP molecule.

Meta-analyses in systematic reviews provide a powerful tool for deriving meaningful conclusions from data of included studies and often help to prevent errors of interpretation [15]. There are, however, pitfalls caused mainly by heterogeneity, of which there are two types: clinical and statistical [27]. Clinical heterogeneity is determined using qualitative measures such as ensuring that trials are similar with respect to patient demographics, study design and outcome measures. If trials are deemed to be homogenous, then their data can be combined in a meta-analysis using either a fixed or a random effects model. In this study, data from 5 *in situ* trials [3,7,8,12,24] that were considered clinically and methodologically homogenous and reported on similar outcomes were pooled for meta-analyses. These results (reflected graphically as forest plots) (Figures 2–4) also provide information on statistical heterogeneity (usually $p < 0.01$) which, if not explained, could render the results of a meta-analysis meaningless. For Figures 2–4, there was significant statistical heterogeneity, which is related to the inconsistency in the size of the treatment effects when the individual trials that were similar in study design, sample size, and outcome measures were compared to each other.

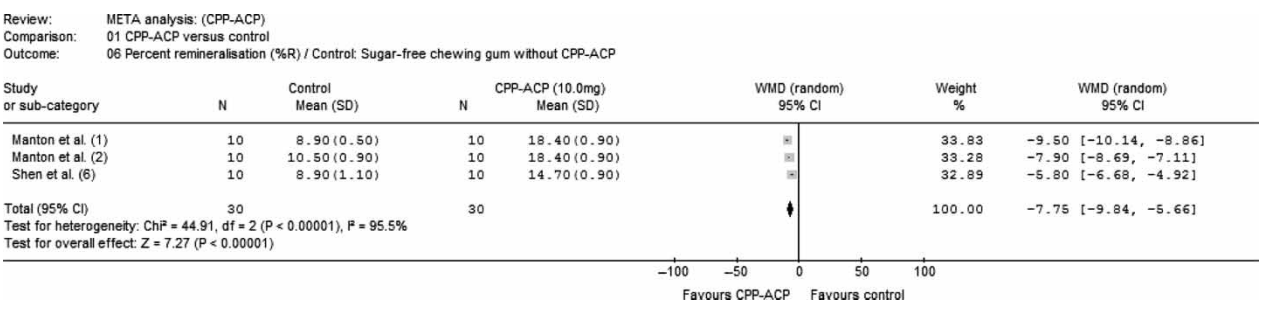

Figure 3. Percentage remineralization (%R) – Subgroup 2. CI = confidence interval; WMD = Weighted mean difference; N = Sample size.

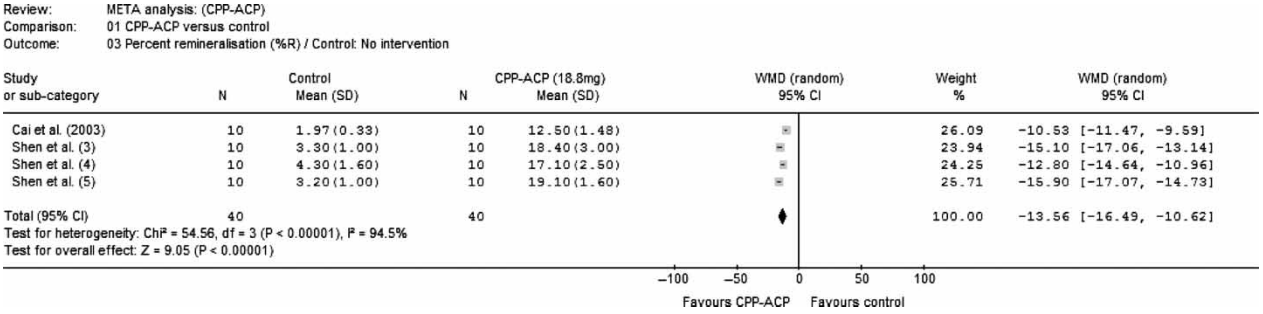

Figure 4. Percent remineralization (%R) – Subgroup 3. CI = confidence interval; WMD = Weighted mean difference; N = Sample size.

The lack of a meta-analysis component in the Azarpazhooh & Limeback systematic review [14] has impacted on the conclusions derived by the authors about the comparative short-term caries-preventive efficacy of CPP-ACP in relation to other interventions. Moreover, this may have led to the error of comparing the number of "positive studies" with the number of "negative studies". According to the Cochrane Handbook [15], such "vote counting" is considered unreliable, "since whether a study is counted as 'positive' or 'negative' may depend on how the results are interpreted by the reviewers and it gives no consideration on the relative weight of reliable evidence contributed by each study". A further report [28] highlighted the tendency to overlook small but clinically important effects when counting votes, particularly when counting studies with statistically insignificant results as 'negative' or 'inconclusive'.

In summary, this review has provided evidence of the short-term and long-term (maximum 24 months) use of CPP-ACP for caries prevention. The dosages found to be effective in short-term trials ranged from 10.0 mg CPP-ACP to 18.8 mg CPP-ACP contained in sugar-free gum. For long-term efficacy (maximum 24 months), a dosage of 54 mg CPP-ACP contained in sugar-free gum was used. The limitations of the *in situ* study design for short-term efficacy should be addressed in future studies by conducting *in vivo* randomized control trials. The outcome measure of such should be clinical caries prevention or caries reduction over a longer (>12 months) period. Reporting of such trials should follow the CONSORT statement [48] and, particularly, include a clear description of how the randomized allocation of study subjects was conducted, report on details of any restrictions, and state who generated the allocation sequence, who enrolled the subjects, and who assigned subjects to their groups. Reporting should further include information about whether such allocation was concealed from the clinical operators until interventions were assigned and, if it was, about how this was done [48].

Within the limitations of this meta-analysis, the results of the *in situ* clinical trials support the short-term remineralization effect of CPP-ACP. Additionally, the *in vivo* randomized clinical trials provide promising results for the long-term use of CPP-ACP for caries prevention. Well-designed *in vivo* randomized clinical trials on the true outcome of caries prevention are warranted.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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